correlation was observed between the expressions of these two molecules (R2 = 0.657, p=0.00121). 35

DETAILED DESCRIPTION OF THE INVENTION

[0037] The invention consists on a vaccine composition able to cause an immune-castration of self-TGFα that can be used for the treatment of certain cancers and other diseases related with TGFα .

[0038] On the other hand this invention include the use of a vaccine preparation constituted by a combination of TGFα and EGF. This vaccine can be used for the treatment of neoplasias that depend on these two growth factors in the course of its pathogenesis.

[0039] 1 - Inmunogenic preparations:

[0040] In the present invention a vaccine preparation used includes the hTGFα either coupled with a carrier protein for methods of genetic engineering (fusion protein) or for chemical methods of conjugation. The hTGFα used in anyone of these immunogenic preparations can be recombinant, synthetic or obtained from natural source. Different proteins can be used as carriers. As examples of carrier proteins can be used: Toxoide Tetanic, KLH, and P64k protein from *Neisseria meningitidis*, among others. The optimum quantity of hTGFα in the vaccine formulation oscillates between 5 μg and 1000 μg per dose.

[0041] On the other hand a vaccine preparation that contains a combination of hTGFα with hEGF (Office of National Registration of Medications, HEBERMIN Not 1266) is used.

[0042] In the specification of TGFα or EGF, any fragment derived from TGFα or EGF that has the same immunology properties and/or similar effects to the original molecule is included. Those derived include, but they are not excluded other, original substitutions of amino acids, change of specific amino acids that increase the stability and/or the activity, chemical modifications, among others.

[0043] A) Obtaining of a fusion protein TGFα -carrier protein by genetic engineering methods:

[0044] The gene coding for hTGFa (500 pb) was amplified by polymerase chain reaction (PCR) using specific primers. The resulting DNA fragment is digested and

cloned in a specific site to an expression vector containing the gene coding for the carrier protein. The resulting protein includes a single or multiple copies of both molecules. You can use an expression vector of mammalian cells, bacteria or yeast. The vector can also include six histidines in the N-terminal end of the carrier protein. The resulting plasmid is verify by restriction analysis on agarosa gels, DNA sequencing using Sequenase 2.0 (Amersham- USB), and finally, analysis of expression of fusion protein in any E.Coli expression strain by Western Blott technique, using an antibody specific monoclonal against hTGFα (R&D System). To obtain the protein the bacterial walls is disrupted using a strong rupture method and then the protein are becomes purified for a combination of differential precipitation methods with ammonium sulfate and chromatography methods. Finally, the protein is filtered under sterile conditions and conserved to - 20°C or lyophilized and conserved at 4°C until its later use.

[0045] B) Obtaining of a chemical conjugated containing hTGFα :

[0046] Different preparations that contain hTGFα conjugated with different carrier proteins (as P64k) are obtained. Any chemical conjugation method can be used. As preferential chemical method is used the method using EMCS agent described in the North American patent, U.S.Pat, Not. 4,302,386; Lee et al., 1981.

[0047] Alternatively, you can use the conjugation method with glutaraldehyde. Briefly, these two or three molecules to a concentration of 1 mg/mL in the final solution are mixing with glutaraldehyde to 0.05% (in the total solution). The mixture is incubated for1 hour at room temperature and then dialyzed against a solution of PBS 1X/10 mM MgCl2. Finally, a dialysis against PBS 1X is carried out overnight at 4°C. The inmunogenic preparation is filter under sterile conditions and stored at 4°C until its use.

[0048] C) Obtaining of a vaccine that combined hTGFα and hEGF.

[0049] The obtaining of a vaccine that combines the two main ligands of the EGF-R can be performed in different ways:

[0050] 1 - Mixing the two vaccines that contain hTGFα or hEGF for separate linked to a carrier protein in a relationship 1:1 just in the moment of the injection. For this purpose can be used the fusion proteins or those chemistry conjugated of each

with a blocking solution of TBS 1X with5% of skim milk overnight at 4 °C. After a brief wash with TBS 1X- Tween 20 (0.05%), membranes were incubated, one replies with an antibody anti-P64K (1 / 500) (Fig.3A) and the other one with a anti-TGFα Mab (1/100) (Fig.3B) for 2 hours at room temperature. Subsequently were performed 3 washes with the same solution and membranes were incubated with alkaline phosphatase-labeled goat anti–mouse immunoglobulins (1/1000) for 1 hour in same conditions. Finally was added 0.004 g of Fast Net enzyme substrate (Sigma) in buffer containig 0.1 M Tris-Cl pH=8.2, 0.004 g of Naphtol ACE-MX Phosphate (Sigma) and 400 µL of NN'Dimetil Formamide in 20 mL. The reaction stopped with similar washes. A specific recognition of TGFα -P64k by the antihTGFα Mab was observed (Fig: 3). This result demonstrates that TGFα in the.fusion protein maintains a structure able of being recognized by a specific antibody.

[0077] Example 7: Obtaining of a chemical conjugated hTGFα -P64k.

[0078] A milliliter of TGFα in PBS/10mM MgCl2 at 2 mg/mL is mixed with a milliliter of P64k at 2 mg/mL in the same solvent. Then 0.2 mL of 0.5% glutaraldehyde solution was added for a final percent of 0.05%. The mixture was incubated 1 hour at room temperature, and dialyzed against a PBS 1X/10 mM MgCl2 solution. Finally, dialysis against PBS 1X was carried out overnight at 4°C. The immunogenic preparation is filtered under sterile conditions and stored at 4°C until its use.

[0079] Example 8: Obtaining of a fusion protein between hTGFα , hEGF and P64k.

[0080] The gene coding for hEGF (150 pb) it is amplified by PCR using the plasmid pBEF 10 as template. That plasmid contains the complete hEGF cloned in the EcoR V site of commercial vector pBluescript SK II (Stragene). The obtained DNA is linked to the pMHisTGFα plasmid in a Bam HI site located in the Cterminal end of the P64k using the methodology described in the example 2. This way the pMTGFα - EGF vector is obtained that codes for the fusion protein TE-P64k.

[0081] Example 9: Obtaining of a chemical conjugated hTGFα -hEGF-P64k.

[0082] A milliliter of TGFα in PBS/10 mM MgCl2 at 3 mg/mL is mixed with a milliliter of hEGF at 3 mg/mL and P64k at 3 mg/mL in the same solvent. Then 0.6 mL